

Genomic DNA Analysis of 15 Inbred Mouse Strains

The NTP evaluates agents of public health concern and maintains a science-based approach to toxicological issues. One issue of practical concern is variability in the toxic response that may be due, in part, to individual genetic differences. Such effects can confound toxicology test results where the response may differ among species, strains and individual animals under the same exposure conditions. Knowing specific genes responsible for variation in susceptibility in test animals could help identify susceptible human genotypes.

Early last year, the NTP prepared a research plan for whole genome DNA sequencing of fifteen inbred mouse strains. Several proposals were received in response to a competitive solicitation to conduct the work, and in September 2004, a contract was awarded to Perlegen Sciences, in Mountain View, California. The study is intended to provide for in the identification of genetic differences, at the nucleotide level, between the 15 strains in comparison with a reference strain (C57BL/6J) that has already been sequenced. High-density oligonucleotide array technology will be used.

The method is based on gene chip technology developed by Affymetrix in which millions of 25mer oligonucleotide probes, created from the known B6 sequence, are covalently bonded to glass chips in large arrays. Each site within an array contains a group of identical oligonucleotides. Families of related probes are synthesized so that the nucleotide in the 13th position is either A, T, G or C. The variable nucleotide is used to query the homologous position in a fragment of fluorescently labeled DNA from a strain to be sequenced. These fragments are hybridized to the chips containing the probes. Binding between probe and target is indicated by fluorescence to indicate which base is present in the 13th position in the target sequence. Confocal laser microscopy is used to measure the fluorescence. The probes are synthesized on the basis of a sliding window, which looks at the known B6 sequence in step-wise fashion. As a result, a base in the 13th position of a probe eventually queries each base of a target sequence.

Perlegen sciences previously demonstrated the technology to discover genomic DNA variability among human subjects. The company has also completed a pilot study, in which it identified DNA variation between 13 classical and two wild-derived inbred mouse strains on subsections of the genome that covered several million bases of DNA. Terms of the contract specify that all data generated will be public ally available through the National Center for Biotechnology Information.

Why the interest in inbred mouse strains? Human beings are enormously variable both in regard to genetic constitution and the environment, which can make it difficult to distinguish between genetic and environmental influences. The inbred strains of mice capture some the same genetic variability that is present in human populations, but within the strains, individual mice are virtually identical, genetically. This characteristic of the inbred strains makes it possible to test the effects of different environmental conditions on different genotypes under controlled laboratory conditions. Therefore, for some environmental diseases, it may be possible to gain specific genetic information pertaining

to susceptibility more efficiently from mice than is presently possible to gain from humans. The project will help provide the means to determine the validity of this concept.

NTP expects Perlegen Sciences to deposit significant amounts of sequence data beginning in March 2005. The project is expected to be complete in September 2006. A website has been established that provides additional technical information regarding the project as well as full and open access to the data (<http://mouse.perlegen.com/mouse/>). As work progresses, investigators who have interest in particular genes will be able to observe the primary nucleotide structure of their genes across 16 mouse strains (including B6) and examine the genes of interest for possible functional alterations.

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